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SOME EFFECTS OF OXYGEN AND CARBON DIOXIDE ON NITRIFICATION AND AMMONIFICATION IN SOILS

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF CORNELL UNIVERSITY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

JAMES KEMP PLUMMER

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SOME EFFECTS OF OXYGEN AND CARBON DIOXIDE ON NITRIFICATION AND AMMONIFICATION IN SOILS[1]

J. K. PLUMMER

Since the theory of nitrification was advanced by Schlœsing and Müntz in 1878, the factors that affect the formation of nitrates and ammonia in soils have been studied rather extensively. It is generally believed that aëration is most commonly the limiting factor, yet there is little conclusive evidence to show whether the effect is due to the specific action of the gases contained in the soil atmosphere. Soils are likely to vary considerably in the relative quantities of oxygen and carbon dioxide held in the pore spaces, this variation depending largely on the relative quantities of easily decomposable organic matter present. The effect of oxygen and carbon dioxide on nitrification and ammonification in soils has been studied to a limited extent, but the evidence obtained seems insufficient to justify any very definite conclusions, as the following review shows.

REVIEW OF EARLIER INVESTIGATIONS

The present theory of nitrification was advanced by Schlœsing and Müntz (1877, a and b, and 1878).[2] These investigators were led to believe that the formation of nitrates is due to some form of life. They state, without presenting data to prove, that there is a direct chemical union of oxygen and nitrogen, brought about by ferments. The oxygen absorbed during the period of nitrification, they say, shows the supply to be sufficient for the oxidation that takes place.

Warington (1892) found that a liberal supply of oxygen is very favorable to nitrification.

Marchal (1893), while working with ammonifying bacteria, found that when the oxygen supply was sufficient some of the ammonifiers were purely aërobic in their behavior. When the conditions became anaërobic, some of these organisms, *Bacillus mycoïdes* especially, behaved as denitrifying bacteria and destroyed the nitrates. Marchal states the conditions best adapted to ammonification, which are thoro aëration, slightly alkaline media, and a temperature of 30° C.

Dehérain (1893) and King and Whitson (1901) compared soil stirred and soil not stirred, and found that nitrates were produced more rapidly in the stirred soils. This they attributed to better aëration.

[1] This investigation was conducted under the direction of Dr. James A. Bizzell.
[2] Dates in parenthesis refer to bibliography, page 328.

Godlewski (1896) observed, from a series of experiments with solutions, that a small amount of carbon dioxide is necessary for the action of the nitrate organism. He found also that the carbon of magnesium carbonate is not suitable for the functions of these bacteria.

Schlœsing (1897) found, after a study of nitrification in clay and in sandy soils, that, contrary to the accepted belief, nitrification went on as rapidly in compact clay soils as it did in coarser-grained soils, when the available water in each case was the same. The experiments of Fischer (1911) led to the same conclusion.

Coleman (1908) found, from a number of experiments, that a supply of carbon dioxide is necessary for both the nitrite and the nitrate organism.

Owen (1908), after a rather extended study of the question, decided that carbon dioxide has no effect on nitrification. In his work nutrient solutions and pure cultures were employed. Air free of carbon dioxide was forced thru 1000-cubic-centimeter flasks containing the solutions. Other flasks contained an atmosphere of pure carbon dioxide.

Stevens and Withers (1909, a and b, and 1910, a and b) concluded from their results that tests made in solutions are not adequate to indicate the behavior of nitrifying or of ammonifying bacteria as compared with their behavior in the soil. These authors state also that nitrification does not take place in sealed flasks.

Effront (1909) showed that ammonification may proceed either in a strictly anaërobic medium with the butyric ferment, or in an aërobic medium in the soil.

Kelley (1911) found, while working with the inundated soils of Hawaii, that ammonia is formed in these soils in their natural state; also, that swamp rice plants prefer this form of nitrogen.

Stoklasa (1912) states that the carbon dioxide evolved can be used as a measure of the bacterial activities in the soil. Thus, where there is greatest nitrification there will be found the greatest production of carbon dioxide.

Temple (1912) determined nitrification in twenty-six Georgia soils. In twenty-four of these, tankage was nitrified more readily than was ammonium sulfate or ammonium chloride. Various other materials produced the same results as did tankage. When calcium carbonate was used, ammonium sulfate and ammonium chloride produced as great a quantity of nitrates as did the other nitrogenous compounds.

SCOPE OF PRESENT WORK

The citations just given will suffice to show the diversity of opinion regarding the relation of oxygen and carbon dioxide to the formation of nitrates and ammonia in soils. The discrepancies are probably due

in many cases to the lack of uniformity in experimental methods. Practically all previous investigators on this subject have used the solution culture method for determining the rate and extent of nitrification and ammonification. This method, however, does not seem suitable for the solution of the problem in question, since the diffusion and absorption of atmospheric gases is not the same as that which takes place when soil is used as the medium.

Again, there seems to be nothing in the recorded data to indicate whether the effects of these gases are specific or whether they are indirect. For example, is carbon dioxide toxic to the nitrification or does its presence merely mean a smaller quantity of oxygen? With the hope of answering questions of this character the following detailed study was undertaken.

RELATION OF OXYGEN AND CARBON DIOXIDE TO NITRIFICATION IN SOILS

Method of investigation

In order to maintain a constant quantity of gas, or even to approximate such a condition, it seemed necessary to conduct the nitrification in hermetically sealed vessels. Otherwise there would be rapid diffusion, and equilibrium would soon be established with gases of the atmosphere. Stevens and Withers (1910 b) state that nitrification does not proceed in sealed flasks. This conclusion is not in harmony with results obtained in the present work, as the data show.

Some preliminary experiments were found necessary in order to determine the best method for preparing the gas mixtures and confining them in the flasks.

In all the experiments 500-cubic-centimeter Erlenmeyer flasks were used. These were fitted with three-hole rubber stoppers, which had been previously boiled in paraffin to prevent absorption of the gases by the rubber. A glass tube bent to serve as a manometer when closed with mercury was placed in one hole of each stopper, so that any change in pressure could be readily observed. The other two holes of the stoppers were fitted with glass tubes, to allow the gases to be forced in and out. (Fig. 66, page 309.) One hundred milligrams of ammonium sulfate was added in solution to 100 grams of fresh soil to which had been added one-half gram of calcium carbonate. Moisture and nitrate determinations were made at the beginning of each experiment, and the water content was brought to 25 per cent, calculated to the dry weight of soil in all cases. Nitrates were determined by the well-known phenol-disulfonic acid method.

The water extracts were made according to the directions given by Schreiner and Failyer (1906). This method consists in adding 500 cubic centimeters of water to 100 grams of soil, stirring for three minutes,

allowing to stand for twenty minutes, and filtering the supernatant liquid through a Pasteur-Chamberlain filter tube.

Oxygen and carbon dioxide were determined by absorption in alkaline pyrogallol and caustic potash, respectively, according to the methods described by Dennis (1913).

The soil used thruout the work was Dunkirk clay loam, taken from under sod directly to the east of the experimental plots on Caldwell Field at Cornell University. This soil was selected because of its low initial nitrate content.

The gas mixtures were made directly in the flasks. The volume of the soil and the water was calculated, and this volume was deducted from the total volume of the flasks. With mercury as the confining liquid, the necessary amount of oxygen to give the desired mixtures was forced in. Previous to adding the oxygen, black rubber gas tubing was placed over the ends of the outlet tubes. After the mixtures were made these were plugged with glass rods. The results of this experiment are given in table 1:

TABLE 1

Flask no.	Composition of gas mixture added		Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Composition of gas mixture at end of experiment	
	Oxygen (per cent)	Nitrogen (per cent)			Oxygen (per cent)	Carbon dioxide (per cent)
1	95	5	14	115.9	30.2	8.4
2	95	5	14	142.7	29.4	9.8
3	95	5	21	204.5	22.4	5.8
4	95	5	21	238.9	20.8	6.0
5	38.2	61.8	14	182.5	23.6	10.9
6	38.2	61.8	14	282.0	12.6	8.3
7	38.2	61.8	21	609.4	10.0	8.0
8	38.2	61.8	21	551.9	5.6	12.0
9	25	75	14	302.9	18.1	8.7
10	25	75	21	493.5	8.3	10.0
11	25	75	21	501.5	8.0	11.6
12	21	79	14	267.1	10.1	5.4
13	21	79	14	232.1	12.0	5.2
14	21	79	21	365.0	12.7	5.9
15	21	79	21	414.5	11.0	6.8

The results obtained from this experiment show that nitrification takes place in closed flasks when calcium carbonate is added with the ammonium sulfate. At the end of fourteen days there was little difference in nitrates formed between the various concentrations of the gas mixtures. At the end of twenty-one days, however, the mixture containing 38.2

per cent of oxygen gave decidedly the highest quantity of nitrates. Just why there should not have been a greater difference due to variations in the oxygen content at the end of fourteen days cannot be explained, unless in those flasks there was greater diffusion thru the rubber connections. In flasks 3 and 4, less carbon dioxide was found at the end of three weeks than in flasks 1 and 2 at the end of two weeks. It seems improbable that there should be so much greater proportionate losses in oxygen content in flasks 1, 2, 3, and 4, with a low nitrate content, than in flasks 7 and 8, which had a higher nitrate content than any in the series. This would suggest that diffusion of the gases had taken place, and it would seem that the greater diffusion had occurred in those flasks that contained the least nitrogen and the greatest amount of oxygen. This is quite reasonable, for in the flasks containing almost pure oxygen there would be a tendency for more nitrogen to diffuse, such flasks acting as a vacuum toward this element.

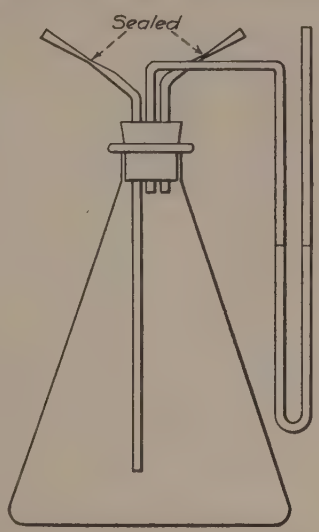

FIG. 66. FLASK USED IN EXPERIMENTS

An experiment was begun to determine whether diffusion occurred thru the rubber connections. In this experiment 95 per cent of oxygen was forced thru flasks fitted similarly to those the results from which are given in table 1 but containing nothing except the oxygen gas. The oxygen was forced thru the flasks for five minutes, at the end of which time samples of the gas leaving the flasks were collected and analyzed. The results are given in table 2:

TABLE 2

Flask no.	Per cent of oxygen at beginning of experiment	Per cent of oxygen at end of experiment	Per cent of oxygen lost
1	94.8	82.8	12.7
2	94.8	82.8	12.7
3	95.0	84.5	11.5
4	95.2	83.9	11.7

These results showed that this method could not be used, for there was a constant interchange of the gases in the flasks with those of the atmosphere.

It was then decided to use glass tubes drawn out to capillary size, and to seal them after the mixtures had been forced thru the flasks until the air had been displaced. The difficulty of getting a sample for analysis when the mixtures were made directly in the flasks made it necessary to devise some other mode of procedure.

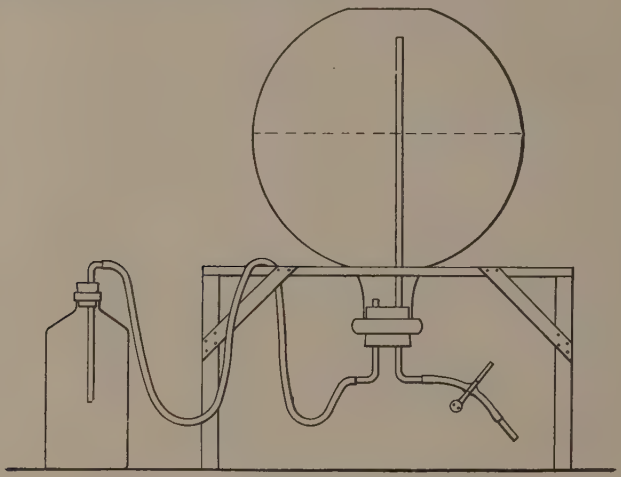

FIG. 67. GASOMETER USED IN EXPERIMENTS

In this experiment it was necessary to ascertain whether there was a loss in the gases thru the rubber stoppers, which had been boiled in paraffin. The exact procedure as given above was repeated, except that the tubes were drawn out and sealed after the flasks had been filled with oxygen (fig. 66). The results are given in table 3:

TABLE 3

Flask no.	Per cent of oxygen at beginning of experiment	Per cent of oxygen at end of 14 days
1	94.8	94.7
2	94.8	94.7
3	95.0	95.0
4	95.0	94.8

These results show that there is no diffusion of oxygen thru the rubber stoppers when they are coated with paraffin. This method of procedure was adopted in all the subsequent work.

The gasometer in which the mixtures were made is shown in figure 67.

Effect of mixtures of oxygen and nitrogen on nitrification

Nitrates produced

It having been found that nitrification would take place in sealed flasks with Dunkirk clay loam when lime was used, experiments were set up to study the effect of oxygen and nitrogen on nitrification. In each of the following experiments 100 grams of fresh soil, screened thru a 3-millimeter sieve, was used. Unless otherwise stated, 0.5 gram of calcium carbonate was mixed with each 100 grams of soil, to make the conditions most favorable for nitrification. The mixing was done all at one time — that is to say, the total amount of soil for the different mixtures was weighed out, and the calcium carbonate was mixed in with this. As in the other experiments, the water content was brought to 25 per cent, calculated to the dry weight of soil. One hundred milligrams of ammonium sulfate was added to the water put in. The soil was spread out on a large glass plate and the solution added in the form of a spray. The soil was then thoroly mixed, and placed in the flasks in 100-gram portions. Mercury was placed in the manometer tubes, which held strips of paper graduated in centimeters.

The gas mixtures were made in the gasometer shown in figure 67, and were forced into the flasks by raising the level bottle. In a number of instances the gas that had passed thru the flasks was analyzed. It was found that by forcing six liters of the gas mixture thru, practically all the atmospheric air had been displaced — this amount being twelve times the volume of the flasks. Rubber tubing that had been boiled in paraffin was placed on the delivery and exit tubes and clamped with stopcocks. The flasks were then placed in the incubator until equilibrium was established in pressure at the new temperature of 30° C. This usually required from ten to fifteen minutes. (It is recognized that there may have been slight diffusion of gases thru the rubber tubing, but this could not be avoided.) The cocks were momentarily opened in order to allow the increase in gas volume due to the rise in temperature to escape, after which the tubes were sealed. The treatments were made in quadruplets, two of which were analyzed at the end of fourteen days and the other two at the end of twenty-eight days. The results, given in tables 4 and 5, are the averages of from two to six determinations for each treatment. All the experiments were repeated at least twice, and some as many as four times. It was not always possible, however, to

obtain exactly the same gas mixture in repeating the treatments. These differences in gas mixtures were small and are given exactly as the analysis showed at the beginning of the experiments. In some cases the difference in nitrate content between the duplicate flasks was greater than was found in those flasks with different gas mixtures, but this was rather unusual. The nitrates are given as increases due to the different treatments. These figures were obtained by subtracting the nitrate content calculated to dry soil, at the beginning, from the total at the end, of the various experiments. This method of calculation was permissible, since the nitrate content in the fresh soil was nearly the same in all cases. The averages of the results obtained with the oxygen-nitrogen mixtures are given in table 4:

TABLE 4

Composition of gas mixture added		Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Composition of gas mixture at end of experiment	
Oxygen (per cent)	Nitrogen (per cent)			Oxygen (per cent)	Carbon dioxide (per cent)
21	79	14	323.1	1.6	9.3
21	79	28	368.8	None.	10.6
30	70	14	388.6	3.8	11.2
30	70	28	499.5	0.7	13.1
40	60	14	727.9	8.1	14.4
40	60	28	957.9	2.1	17.1
45	55	14	707.8	15.0	12.2
45	55	28	900.8	7.6	16.7
50	50	14	590.0	22.7	11.8
50	50	28	742.9	16.4	15.8
55	45	14	469.3	33.7	9.1
55	45	28	815.7	15.8	15.6
60	40	14	355.5	40.2	9.1
60	40	28	725.9	27.8	14.9
70	30	14	290.9	52.5	9.1
70	30	28	661.9	45.0	14.9
80	20	14	232.4	68.2	7.7
80	20	28	346.8	60.1	13.8
85	15	14	219.3	71.7	8.3
85	15	28	336.1	65.9	13.4
95	5	14	249.9	86.2	5.2
95	5	28	271.3	78.1	10.5

From the results shown in this table it can readily be seen that oxygen has a stimulating effect on nitrification. Beginning with the mixture containing 21 per cent of oxygen and 79 per cent of nitrogen (ordinary air), there are marked increases in the production of nitrates as the oxygen supply is increased until 40 per cent is reached. This holds good for both fourteen and twenty-eight days incubation. With 50 per cent of

oxygen there are somewhat less nitrates formed than with 45 per cent of oxygen. As the supply of oxygen is increased beyond this point there is a rather regular decrease in nitrate production. It would thus seem that the gas mixtures containing from 40 to 45 per cent of oxygen are the most favorable to nitrification in this soil. These results show that the higher amounts of oxygen, while depressing the formation of nitrates, do not entirely prevent it. There are 73.2 parts per million less nitrates in the flasks containing 95 per cent of oxygen at the end of fourteen days than in the flasks containing ordinary air. The difference for

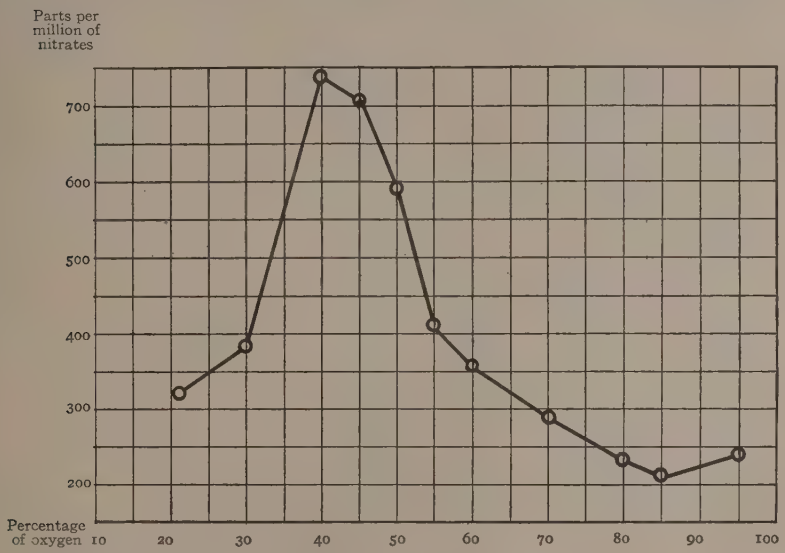

FIG. 68. PRODUCTION OF NITRATES WITH INCREASE IN OXYGEN CONTENT, AT THE END OF FOURTEEN DAYS

twenty-eight days is 97.5 parts per million. This is not what would be expected, for it would seem that the nitrates produced during the first fourteen days would rarify the oxygen somewhat, and thus, the oxygen being further diluted with carbon dioxide, better conditions for nitrification would be obtained. There is no apparent explanation for this depressing effect.

One noticeable point shown by the composition of the mixtures at the end of fourteen and of twenty-eight days is the large consumption of oxygen. In the flasks containing 21 per cent of oxygen at the beginning, there was only 1.6 per cent at the end of fourteen days and none at the

end of twenty-eight days. In the flasks originally containing 40 per cent of oxygen, there was only 8 per cent and 2 per cent at the end of fourteen and of twenty-eight days, respectively. This loss of oxygen is always greater than the carbon dioxide produced, and increases regularly with the nitrates formed. The production of nitrates with increases in the oxygen content of the various gas mixtures at the end of fourteen days is shown graphically in figure 68.

The preceding results were obtained with soil to which ammonium sulfate and calcium carbonate had been added. It was decided to try oxygen and nitrogen mixtures with the fresh soil, without the addition of ammonium sulfate or calcium carbonate. The results are given in table 5:

TABLE 5

Composition of gas mixture added		Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Composition of gas mixture at end of experiment	
Oxygen (per cent)	Nitrogen (per cent)			Oxygen (per cent)	Carbon dioxide (per cent)
21	79	14	99.3	8.2	6.1
21	79	14	95.2	9.6	5.9
31	69	14	117.6	14.5	7.6
31	69	14	117.6	15.6	8.8
43	57	14	152.4	22.0	10.5
43	57	14	194.8	21.8	10.1
48	52	14	147.0	29.3	10.6
48	52	14	152.4	27.7	11.0
52	48	14	135.5	32.5	9.5
52	48	14	148.9	34.3	9.8
58	42	14	117.6	42.3	8.9
58	42	14	108.6	43.7	9.4
63	37	14	95.2	48.3	10.0
63	37	14	95.2	49.1	8.8
69	31	14	95.2	53.1	8.9
69	31	14	95.2	52.2	9.0
76	24	14	83.0	62.3	8.6
76	24	14	83.0	61.6	8.8
93.5	6.5	14	53.9	84.0	4.3
93.5	6.5	14	50.4	85.1	3.8

In this case, as in the experiments in which lime and ammonium sulfate were added, there is a stimulating effect on nitrification caused by an increase in the oxygen content of the atmosphere in the flask. This increase is greatest in the mixtures containing from 43 to 52 per cent of oxygen. Tho the degree of nitrification is much less when there is no calcium carbonate nor ammonium sulfate, the differences in the two experiments run parallel.

The quantity of carbon dioxide evolved is also lower than in the corresponding experiments in which lime was used. This would tend to show that a basic condition is best suited for the production of this gas.

Relation of oxygen consumed to nitrates formed

The amount of oxygen not accounted for by the carbon dioxide formed plus the oxygen at the end of the experiment, was calculated to parts per million of oxygen (by weight) lost per 100 grams of dry soil. The weight of oxygen corresponding to that which went into the nitrates was calculated for the different mixtures. The results of these calculations, which were made from the averages of the oxygen-nitrogen treatments shown in table 4, are given in table 6:

TABLE 6

Composition of gas mixture added		Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Per cent of oxygen not recovered as such	Parts per million of oxygen and nitrogen found	Parts per million of oxygen not recovered as such
Oxygen (per cent)	Nitrogen (per cent)					
21	79	14	323.1	10.3	250.4	165.2
21	79	28	368.8	10.4	285.8	166.4
30	70	14	388.6	14.8	301.6	321.9
30	70	28	499.5	16.3	387.1	372.8
40	60	14	727.9	17.1	564.1	522.2
40	60	28	957.9	20.8	742.4	635.3
45	55	14	707.8	17.8	548.5	611.5
45	55	28	900.8	20.7	698.1	711.2
50	50	14	590.0	13.0	457.2	496.4
50	50	28	742.9	17.8	575.4	680.6
55	45	14	409.3	13.4	317.2	562.7
55	45	28	815.7	20.1	630.0	844.2
60	40	14	355.5	10.7	275.4	490.3
60	40	28	725.9	17.3	562.4	793.9
70	30	14	290.9	6.8	225.1	369.2
70	30	28	661.9	10.1	512.9	531.6
80	20	14	232.4	4.1	180.9	250.5
80	20	28	346.8	6.2	268.3	378.8
85	15	14	219.3	3 0	169.9	194.6
85	15	28	336.1	5.7	252.1	369.8
95	5	14	249.9	3.8	193.6	275.6
95	5	28	271.3	6.9	200.3	500.4

It can readily be seen from the calculations given in table 6 that the oxygen consumed during the processes that went on in the flasks was greater than that required to form nitrates. With four exceptions this was the case, and these occurred where the oxygen supply was found to be limited. It seems to the writer that these results are consistent and that the excess is great enough to justify the conclusion that oxygen

is used in other processes than in forming nitrates. The amount of oxygen not recovered by the analyses either as oxygen or as carbon dioxide is greatest when the nitrate production is at its maximum.

The results given in table 6 are shown graphically in figure 69.

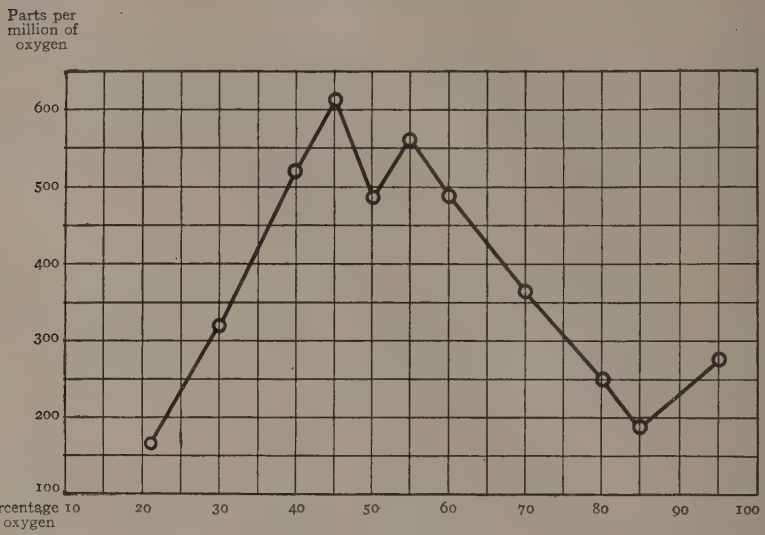

FIG. 69. OXYGEN FIXED BY BACTERIA WITH INCREASE IN OXYGEN CONTENT, AT THE END OF FOURTEEN DAYS

Relation of carbon dioxide produced to nitrates formed

Another interesting point brought out by table 4 is the production of carbon dioxide. The amount formed increases fairly regularly with the nitrates produced. This is in line with the work of Stoklasa (1912), who states that the carbon dioxide produced can be used as a measure of bacterial activity in soils. The amount of carbon dioxide formed as shown in table 4 can be better illustrated by computing the percentages found to parts per million formed by 100 grams of soil. This is done in a manner similar to that followed in making the oxygen calculations, computing first the volume occupied by the gas mixtures and then the volume of carbon dioxide produced. One cubic centimeter of carbon dioxide weighs .00198 gram. The results of such calculations taken from table 4 are given in table 7.

Tho ammonium sulfate was used as the nitrifiable material, the uniform increase of carbon dioxide formed with nitrates produced is entirely reasonable. The conditions in the flasks were the best possible for bacterial

TABLE 7

Composition of gas mixture added		Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Parts per million carbon dioxide produced by 100 grams of soil
Oxygen (per cent)	Nitrogen (per cent)			
21	79	14	323.1	998.5
21	79	28	368.8	1,135.9
30	70	14	388.6	1,200.0
30	70	28	499.5	1,403.0
40	60	14	727.9	1,543.0
40	60	28	957.9	1,832.0
45	55	14	707.8	1,307.5
45	55	28	900.8	1,789.6
50	50	14	590.0	1,265.6
50	50	28	742.9	1,693.2
55	45	14	409.3	978.6
55	45	28	815.7	1,671.7
60	40	14	355.5	978.6
60	40	28	725.9	1,596.7
70	30	14	290.9	978.6
70	30	28	661.9	1,596.7
80	20	14	232.4	824.4
80	20	28	346.8	1,278.8
85	15	14	219.3	889.6
85	15	28	336.1	1,462.0
95	5	14	249.9	557.2
95	5	28	271.3	1,068.0

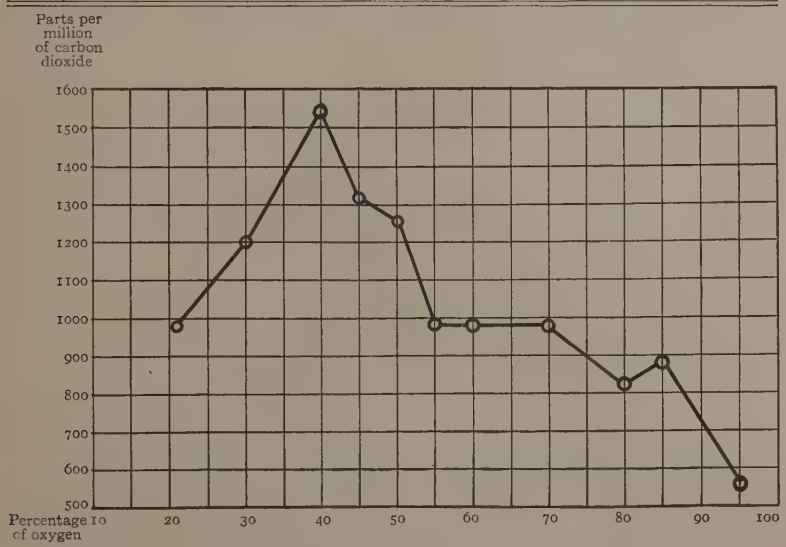

FIG. 70. PRODUCTION OF CARBON DIOXIDE WITH INCREASE IN OXYGEN CONTENT, AT THE END OF FOURTEEN DAYS

activities, and undoubtedly some of the nitrogen of the organic content of the soil was nitrified; hence the destruction of the organic matter. It is, then, reasonable to expect that where there is the greatest decomposition of such material — and such must be the case before the nitrogen can be converted to nitrates — there will be the greatest quantity of carbon dioxide formed.

The results shown in table 7 are plotted in figure 70.

Effect of mixtures of carbon dioxide and nitrogen on nitrification

The influence of oxygen-nitrogen mixtures on nitrification having been studied, it was decided to ascertain what would be the effect of mixtures of carbon dioxide and nitrogen. The nitrogen was obtained by forcing ordinary air thru a series of gas-washing bottles containing alkaline pyrogallol to remove the oxygen. In this way a very pure form of nitrogen was obtained, in some cases analyzing as high as 99.2 per cent nitrogen.

The results of an experiment with these mixtures are given in table 8. This experiment was repeated with essentially the same results.

TABLE 8

Composition of gas mixture added			Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Composition of gas mixture at end of experiment	
Oxygen (per cent)	Carbon dioxide (per cent)	Nitrogen (per cent)			Oxygen (per cent)	Carbon dioxide (per cent)
21	..	79	14	161.3	3.4	7.1
21	..	79	14	168.0	4.1	6.9
..	73	27	14	—14.6*	None	72.8
..	73	27	14	—14.6	None	73.2
..	61	39	14	—14.6	None	60.0
..	61	39	14	—14.6	None	60.5
..	52	48	14	—14.6	None	51.6
..	52	48	14	—14.6	None	51.7
..	40	60	14	—14.6	None	40.0
..	40	60	14	—14.6	None	39.8
..	30	70	14	—14.6	None	29.7
..	30	70	14	—14.6	None	29.8
..	24	76	14	—14.6	None	24.2
..	24	76	14	—14.6	None	23.9
..	99	..	14	—14.6	None	0.8
..	99	..	14	—14.6	None	0.6

*Minus sign denotes that there were less nitrates than at the beginning of the experiment.

From the results presented in table 8 it is quite evident that the mixtures composed of carbon dioxide and nitrogen are not favorable to nitrate formation in soils. There were 14.6 parts per million of nitrates at the beginning of the experiment, and in all cases there was only a trace at the end. The denitrifying bacteria apparently destroyed the nitrates present at the beginning.

Effect of mixtures of carbon dioxide and oxygen on nitrification

Certain concentrations of oxygen mixed with nitrogen appeared to increase the formation of nitrates beyond that which takes place in the atmosphere. The question arose, Does this hold true for oxygen when in association with carbon dioxide? Generally it is considered that the inert gas nitrogen plays no part in the actual formation of nitrates. Any appreciable variation between the results obtained with the same concentrations of oxygen diluted with nitrogen and with carbon dioxide, must be due to the latter gas. The experiments to ascertain the effect of mixtures of carbon dioxide and oxygen were conducted exactly as were those with the oxygen-nitrogen mixtures. The carbon dioxide used was of the highest purity, analyzing 99.8 per cent pure. The oxygen used contained 5 per cent of nitrogen. The average results obtained from these experiments are given in table 9.

It appears from table 9 that the maximum nitrate production resulted with the mixture composed of 60 per cent of carbon dioxide and 35 per cent of oxygen. With the oxygen-nitrogen mixtures the maximum quantity of nitrates was produced by the mixtures containing from 40 to 45 per cent of oxygen (table 4). Taking into account the experimental error, these results are therefore in close agreement with those of the experiments with the oxygen-nitrogen mixtures.

A point of interest brought out in table 9 is the lower amount of nitrates produced at the end of twenty-eight days with the mixture containing 75 per cent of carbon dioxide and 20 per cent of oxygen than was found at the end of fourteen days. A glance at the composition of the mixture at the end of each experiment shows that the oxygen supply was very low at the end of fourteen days — only 2 per cent — while at the end of twenty-eight days there was no oxygen. After this point is reached in the mixtures, as the amount of carbon dioxide increased the amount of nitrates produced decreased. In the mixture containing 90 per cent of carbon dioxide there were no nitrates formed, but those present at the beginning were destroyed. This indicates that denitrification had set in with the mixtures containing a low percentage of oxygen. Bréal (1892) and others have observed that the denitrifiers show their effect when the oxygen supply is low.

In general these results show little difference from those obtained with the oxygen-nitrogen mixtures as long as there is a supply of oxygen present. So it seems to be of little consequence whether the oxygen is diluted with nitrogen or with carbon dioxide for the production of nitrates in soils.

TABLE 9

Composition of gas mixture added			Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Composition of gas mixture at end of experiment	
Carbon dioxide (per cent)	Oxygen (per cent)	Nitrogen (per cent)			Oxygen (per cent)	Carbon dioxide (per cent)
0.8	94.8	5.0	14	250.0	83.3	9.0
0.8	94.8	5.0	28	277.0	77.7	12.6
2.0	93.0	5.0	14	286.0	79.0	12.0
2.0	93.0	5.0	28	322.2	76.1	14.6
4.0	91.0	5.0	14	295.5	75.6	14.4
4.0	91.0	5.0	28	377.7	70.9	18.6
9.6	85.4	5.0	14	317.8	70.6	20.1
9.6	85.4	5.0	28	499.0	64.3	24.2
15.0	80.0	5.0	14	349.9	64.7	25.2
15.0	80.0	5.0	28	533.0	51.8	29.8
20.0	75.0	5.0	14	328.3	62.5	26.8
20.0	75.0	5.0	28	644.5	53.0	33.3
25.0	70.0	5.0	14	347.5	55.4	33.1
25.0	70.0	5.0	28	693.1	47.5	38.9
31.0	64.0	5.0	14	371.9	48.3	39.5
31.0	64.0	5.0	28	693.1	36.3	44.0
41.0	54.0	5.0	14	537.0	29.9	54.7
41.0	54.0	5.0	28	763.4	23.6	57.8
45.0	50.0	5.0	14	539.5	24.8	58.4
45.0	50.0	5.0	28	798.4	16.9	61.9
51.0	44.0	5.0	14	596.4	17.5	64.6
51.0	44.0	5.0	28	846.7	9.4	67.2
60.0	35.0	5.0	14	671.1	2.9	75.8
60.0	35.0	5.0	28	896.5	None	80.0
65.0	30.0	5.0	14	511.1	4.7	67.2
65.0	30.0	5.0	28	624.8	None	79.6
75.0	20.0	5.0	14	485.8	2.0	81.0
75.0	20.0	5.0	28	405.1	None	85.2
80.0	15.0	5.0	14	337.9	1.4	87.9
80.0	15.0	5.0	28	299.1	None	86.3
90.0	5.0	5.0	14	—36.5*	None	93.9
90.0	5.0	5.0	28	—30.1	None	94.1
93.0	2.0	5.0	14	—29.4	None	96.1
93.0	2.0	5.0	28	—36.5	None	95.6
99.0	....	....	14	—36.5	None	98.8
99.0	....	....	28	—36.5	None	99.2

*Minus sign denotes that there were less nitrates than at the beginning of the experiment.

Effect of mixtures of carbon dioxide, oxygen, and nitrogen on nitrification

Since the soil air is made up of various concentrations of oxygen, nitrogen, and carbon dioxide, varying mixtures of these gases were used in further study. The results are given in table 10.

TABLE 10

Composition of gas mixture added			Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Composition of gas mixture at end of experiment	
Carbon dioxide (per cent)	Oxygen (per cent)	Nitrogen (per cent)			Oxygen (per cent)	Carbon dioxide (per cent)
0.5	20.9	78.7	14	382.4	1.0	11.2
0.5	20.9	78.7	28	452.1	None	13.8
1.0	20.8	78.7	14	423.7	1.2	12.5
1.0	20.8	78.7	28	480.0	None	14.9
2.5	20.5	77.0	14	423.7	1.0	14.2
2.5	20.5	77.0	28	547.0	None	16.7
5.0	20.0	75.0	14	366.5	0.9	15.4
5.0	20.0	75.0	28	478.7	None	19.4
8.0	19.3	72.7	14	340.1	0.6	17.7
8.0	19.3	72.7	28	374.1	None	24.0
10.0	19.0	71.0	14	315.3	0.6	17.7
10.0	19.0	71.0	28	372.8	None	19.7
15.0	17.9	67.1	14	304.4	0.5	21.0
15.0	17.9	67.1	28	337.9	None	24.0
20.0	16.8	63.6	14	282.2	0.1	26.0
20.0	16.8	63.6	28	324.4	None	26.8
30.0	14.7	55.3	14	210.0	0.2	33.8
30.0	14.7	55.3	28	310.7	None	36.8
35.0	13.4	51.1	14	174.0	None	38.8
35.0	13.4	51.1	28	240.6	None	39.8
44.0	11.8	45.0	14	163.3	None	47.7
44.0	11.8	45.0	28	86.1	None	46.5
49.0	10.7	40.3	14	166.6	None	47.7
49.0	10.7	40.3	28	70.2	None	46.5
55.0	9.5	35.3	14	9 2	None	58.8
55.0	9.5	35.3	28	— 8.3*	None	63.1
60.0	8.4	31.6	14	10.3	None	63.9
60.0	8.4	31.6	28	— 8.6	None	68.7
69.0	6.5	24.3	14	— 5.5	None	74.6
69.0	6.5	24.3	28	— 9.9	None	75.2
77.0	4.6	18.4	14	— 7.6	None	82.1
77.0	4.6	18.4	28	— 8.8	None	81.1
86.0	2.9	11.1	14	—14.3	None	88.3
86.0	2.9	11.1	28	— 9.1	None	88.6
98.0	0.4	1.6	14	—22.3	None	98.6
98.0	0.4	1.6	28	—22.3	None	98.7

*Minus sign denotes that there were less nitrates than at the beginning of the experiment.

The lower concentrations of carbon dioxide seem to have little effect on nitrification as shown in table 10. Increasing the amount of carbon dioxide above 30 per cent causes a noticeable reduction in nitrates formed. So long as the oxygen supply is about the same, the variations are slight, giving further evidence that oxygen is necessary to promote nitrification in soils. After the 30-per-cent limit of carbon dioxide is passed, no oxygen is found in the gas mixtures even after fourteen days of incubation. A steady decrease in nitrates is also noted beyond this limit, and when 44

per cent of carbon dioxide is reached there are less nitrates at the end of twenty-eight days than were found at fourteen days. Beyond 60 per cent of carbon dioxide denitrification occurred in all cases. Tho the nitrates were destroyed, none of the oxygen was liberated in the elementary form, for in no case was any oxygen found in the gas mixtures.

In Owen's (1908) work, in which nitrate tests were supposed to be conducted in an excess of carbon dioxide, there really was an atmosphere entirely composed of this gas. To find no increase in nitrate formation under such conditions is exactly what is to be expected.

Denitrification occurred where there was a limited supply of oxygen, and it was thought to be of interest to get further data as to the extent to which nitrates were destroyed by the denitrifying bacteria. In this experiment eight flasks were set up as usual, with ordinary air instead of gas mixtures. Two of these flasks were analyzed at the end of fourteen days, two at the end of twenty-eight days, and the remaining four at the end of fifty-six days. The results are given in table 11:

TABLE 11

Composition of gas mixture added		Time of incubation (days)	Nitrates (NO_3) (parts per million dry soil)	Composition of gas mixture at end of experiment	
Oxygen (per cent)	Nitrogen (per cent)			Oxygen (per cent)	Carbon dioxide (per cent)
21	79	14	290.8	2.7	10.2
21	79	14	308.8	2.1	11.0
21	79	28	353.9	None	11.9
21	79	28	353.9	None	11.8
21	79	56	—19.6*	None	17.3
21	79	56	—18.4	None	18.8
21	79	56	—18.6	None	19.0
21	79	56	—20.7	None	19.0
Nitrates at beginning			23.1		
Nitrates at beginning			23.1		

*Minus sign denotes that there were less nitrates than at the beginning of the experiment.

The results show clearly that the bacteria themselves can produce conditions favorable for denitrification. The percentage of oxygen found in the flasks that were analyzed at the end of fourteen days was only 2.4. At the end of twenty-eight days there was no oxygen, but the nitrates were somewhat higher than at fourteen days. In the flasks that were incubated for fifty-six days there were found less nitrates than the soil contained at the beginning of the experiment. This is in agree-

ment with the work of Dehérain (1897), who found that denitrification was an extremely rapid process when the conditions were favorable to the denitrifying bacteria. Lemmermann, Fischer, Kappen, and Blanck (1909) observed that lime increases the action of the denitrifiers. The conditions were therefore favorable for this process in this experiment. Here again none of the oxygen that was consumed in the production of nitrates was found in the elementary form.

RELATION OF OXYGEN AND CARBON DIOXIDE TO AMMONIFICATION IN SOILS

Method of investigation

Casein was chosen as the substance to furnish the ammonifiable nitrogen, and its solution in sodium hydroxide was made according to the directions given by Brown (1913). Moisture was determined at the beginning of each experiment and 100 grams of fresh soil was placed on a large glass plate. Ten cubic centimeters of the casein solution, with enough water added to bring the water content to 25 per cent (dry basis), was intimately mixed with the soil. This was then placed in the flasks (500-cubic-centimeter Erlenmeyer) and exposed to the various gas mixtures for three days at a temperature of 30° C. At the end of three days the flasks were removed and 400 cubic centimeters of water, containing 4 cubic centimeters of concentrated hydrochloric acid, was added. This was stirred for five minutes and allowed to set for twenty minutes, and the whole was then thrown on an ordinary filter. Three hundred cubic centimeters of the filtrate was distilled with magnesium oxide, the distillate being caught in fifth-normal hydrochloric acid and titrated in the usual manner.

It was found that there was practically no difference between the results obtained with varying mixtures of oxygen and nitrogen, as will be shown later. Since it was possible that the ammonia was produced in the distillation process, experiments were set up to test this point. A comparison of the different methods of distillation was also made — that is, distillation of the soil directly with magnesium oxide by the use of steam, and extraction with weak hydrochloric acid. This was done as follows: To eight 100-gram portions of fresh soil 10 cubic centimeters of casein solution was added. Four of these portions were extracted with dilute hydrochloric acid and the filtrate was distilled with magnesium oxide, as usual. The other four were washed into the distillation flasks, magnesium oxide was added, and the distillation was made with steam. The results of this experiment are given in table 12:

TABLE 12

Extraction with hydrochloric acid		Distillation with steam	
Flask no.	Milligrams of ammonia nitrogen per 100 grams of soil	Flask no.	Milligrams of ammonia nitrogen per 100 grams of soil
1	0.39	1	1.12
2	0.56	2	0.84
3	0.93	3	0.84
4	0.56	4	0.64
Average	0.61	Average	0.86

These results show that the ammonia produced is not developed in the process of distillation. As the extraction method showed slightly lower results than distilling the soil directly, it was decided to continue its use.

It was thought probable that nitrification had taken place to some extent during the three days of incubation. Stevens and Withers (1910 b) and others have found that even excessive quantities of organic matter do not inhibit nitrification in soils.

In order to determine nitrates in the presence of excessive quantities of organic matter, it is necessary to use some other than the phenol-disulfonic-acid method, it being generally recognized that this method is not reliable in such cases. Burgess (1913) showed that the aluminum reduction method may be satisfactorily applied to soils containing alkali salts. It appears that this method is applicable for the determination of nitrates in the presence of organic material. It is in common use for such determination with sewage waters, in which the organic content is high.

Ammonia and nitrate determinations could not be made on the same sample. It was therefore necessary to set up four flasks, in two of which ammonia was determined by the extraction method while the other two were analyzed for nitrates by the aluminum reduction method.

Effect of mixtures of oxygen and nitrogen on ammonification

The averages of three experiments with the same gas mixtures are given in table 13.

Except in the case of 95-per-cent oxygen there is little difference in the amounts of ammonia formed. This result is rather unexpected, since the various oxygen-nitrogen mixtures produce decidedly different

effects on nitrification. The analyses of the gases show clearly that oxygen has been consumed with the rapid production of carbon dioxide. An interesting point brought out in this connection is the rapidity with which anaërobic conditions are produced, even with mixtures containing as much as 32 per cent of oxygen. Just why there should be so much less carbon dioxide found with the higher oxygen contents is not clear, unless there is less bacterial activity with these concentrations.

TABLE 13

Composition of gas mixture added		Milligrams of ammonia nitrogen per 100 grams of soil	Composition of gas mixture at end of experiment	
Oxygen (per cent)	Nitrogen (per cent)		Oxygen (per cent)	Carbon dioxide (per cent)
21	79	21.0	None	16.3
32	68	18.8	None	26.5
40	60	19.9	3.2	28.2
50	50	18.6	7.1	34.5
75	25	16.2	33.3	22.6
95	5	10.2	73.5	20.7

Experiments were set up to ascertain whether nitrification had taken place in the flasks. The averages of three experiments to test this point are given in table 14:[3]

TABLE 14

Composition of gas mixture added		Milligrams of nitrogen as NH_3 per 100 grams of soil	Milligrams of nitrogen as NO_3 per 100 grams of soil	Total milligrams of nitrogen ammonified per 100 grams of soil
Oxygen (per cent)	Nitrogen (per cent)			
21	79	16.4	2.8	19.2
30	70	17.6	3.3	20.9
40	60	17.5	4.2	21.7
50	50	17.7	4.6	22.3
60	40	17.5	4.2	21.7
80	20	16.6	3.0	19.6
90	10	14.5	2.6	17.1
95	5	11.4	1.9	13.3

The table shows that nitrification does take place in the flasks even tho the period of incubation is short. Whether this nitrification is as great

[3] The nitrate nitrogen reported in this table is the amount formed during incubation.

as is represented by the analyses the writer will not attempt to say. Qualitative tests by the diphenylamine reaction show that there are considerably more nitrates present after incubation than when incubation was begun. Nitrates were determined by the phenol-disulfonic-acid method at the beginning of each experiment and the amount found was deducted from the amount found by the reduction method at the end, giving the figures shown in the table. There is a rather close agreement in the rise of nitrate nitrogen with that produced in the nitrification studies (table 4). The results from both experiments show the maximum amount with mixtures containing from 40 to 50 per cent of oxygen.

The nitrogen occurring as ammonia is nearly the same in all cases, except with the pure oxygen. Including the pure oxygen, there is a difference of only six milligrams between the least and the greatest amount produced. When the total nitrogen ammonified (nitrate nitrogen plus ammonia nitrogen) is taken into account the difference is greater, being in this case 9 milligrams. This indicates a tendency toward equilibrium between the nitrifiers and the ammonifiers. Assuming that nitrates must pass through the ammonia stage, the total ammonia produced is increased by a supply of oxygen. The mixtures containing from 30 to 80 per cent of oxygen give consistently higher results than does ordinary air. The basic condition of the soil used in these experiments tended to increase the activities of all bacteria operating in the flasks.

Effect of mixtures of oxygen and carbon dioxide on ammonification

The mixtures used in the experiments to determine the effect of oxygen and carbon dioxide on ammonification were made as in the nitrification experiments (page 319). The averages of the results are given in table 15:

TABLE 15

Composition of gas mixture added			Time of incubation (days)	Milligrams of nitrogen as NH_3 per 100 grams of soil	Milligrams of nitrogen as NO_3 per 100 grams of soil	Total milligrams of nitrogen ammonified per 100 grams of soil
Oxygen (per cent)	Nitrogen (per cent)	Carbon dioxide (per cent)				
21	79	...	3	18.6	2.0	20.6
21	79	...	3	18.6	2.2	20.8
40	....	60	3	18.0	3.8	21.8
40	...	60	3	18.6	3.2	21.8
60	...	40	3	18.4	3.6	22.0
60	...	40	3	18.1	3.6	21.7
70	...	30	3	17.2	3.0	20.2
70	...	30	3	16.8	2.8	19.6
90	...	10	3	12.8	2.0	14.8
90	...	10	3	13.0	1.8	14.8

While there are little differences in ammonia nitrogen between the various gas mixtu es shown in the above table, there is a slight increase in nitric nitrogen with the mixtures containing from 40 to 60 per cent of oxygen. This was found to be the case also with the oxygen-nitrogen mixtures. It would seem, therefore, to be of small consequence whether the oxygen is diluted with carbon dioxide or with nitrogen, so long as there is a liberal supply present.

Effect of mixtures of carbon dioxide, oxygen, and nitrogen on ammonification

The results of the experiment to determine the effect of various mixtures of carbon dioxide, oxygen, and nitrogen on ammonification are given in table 16:

TABLE 16

Composition of gas mixture added			Milligrams of nitrogen as NH_3 per 100 grams of soil	Milligrams of nitrogen as NO_3 per 100 grams of soil	Total milligrams of nitrogen ammonified per 100 grams of soil
Carbon dioxide (per cent)	Oxygen (per cent)	Nitrogen (per cent)			
10.0	18.9	71.1	17.5	5.9	23.4
20.0	16.8	63.2	16.9	5.9	22.8
40.0	12.6	47.4	17.0	4.4	21.4
60.0	8.4	31.6	16.4	4.2	20.6
80.0	4.2	15.8	13.8	1.5	15.3
99.0	....	....	9.8	0.3	10.1

Here again nitrification appears plainly. Tho the differences are small until the higher concentrations of carbon dioxide are reached, they are perceptible. The lowering of the amount of ammonia produced in the presence of high concentrations of carbon dioxide is noteworthy. That any appreciable quantity of ammonia is formed under strictly anaërobic conditions is in line with the work of Kelley (1911) on the inundated soils of Hawaii. In general the results are in agreement with those already shown. There are less ammonia and nitrates formed when the carbon dioxide is highest and the oxygen lowest. This necessity for oxygen is indicated also in the results from the mixtures of carbon dioxide and nitrogen (table 8).

SUMMARY

Altho the methods used in this investigation are not so suitable as might be desired, the following conclusions seem to be justified from the results obtained:

1. Vigorous nitrification takes place in sealed flasks as long as there is a supply of oxygen.

2. Of the soil gases studied, oxygen is the limiting constituent, and there is an optimum mixture of this gas (one containing from 35 to 60 per cent of oxygen) for nitrification.

3. From the losses of oxygen from the gas mixtures it seems certain that there are other forms of oxidation than that caused by the nitrate bacteria.

4. A large quantity of carbon dioxide is produced when lime is used, amounting in some instances to nearly 20 per cent. The greatest production of this gas accompanies the point of maximum nitrification. Ammonium sulfate, when applied to this soil without the addition of lime, produces only slight increases in nitrification even after incubation for a period of twenty-eight days. The small quantity of carbon dioxide formed under such conditions would tend to indicate that ammonium sulfate, when applied to this soil alone, depresses the action of the carbon-dioxide-producing bacteria as well.

5. Taking the results as a whole it cannot be said that carbon dioxide has any material effect on nitrification so long as oxygen is present in the atmosphere. It is of little consequence whether the oxygen is diluted with the inert gas nitrogen or with carbon dioxide. When the supply of oxygen becomes limited and anaërobic conditions are produced, denitrification sets in, and this continues until practically all the nitrates are destroyed. In no case with these experiments was the combined oxygen liberated in the elementary form during the processes of denitrification.

6. The results with the distillation method for the determination of ammonia show that there is no optimum content of oxygen for the production of this compound. The results of all mixtures, except the very high concentrations of oxygen, are practically the same. It seems to make little difference whether the mixtures are made with oxygen and nitrogen or with oxygen and carbon dioxide. Under purely anaërobic conditions, caused by an atmosphere of pure carbon dioxide, there is somewhat less ammonia produced than when oxygen is present at the beginning. That ammonia is formed in rather large quantities under such conditions is noteworthy.

7. Nitrate determinations show that nitrates are produced in ammonification tests even tho the organic content is high and the period of incubation is short.

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